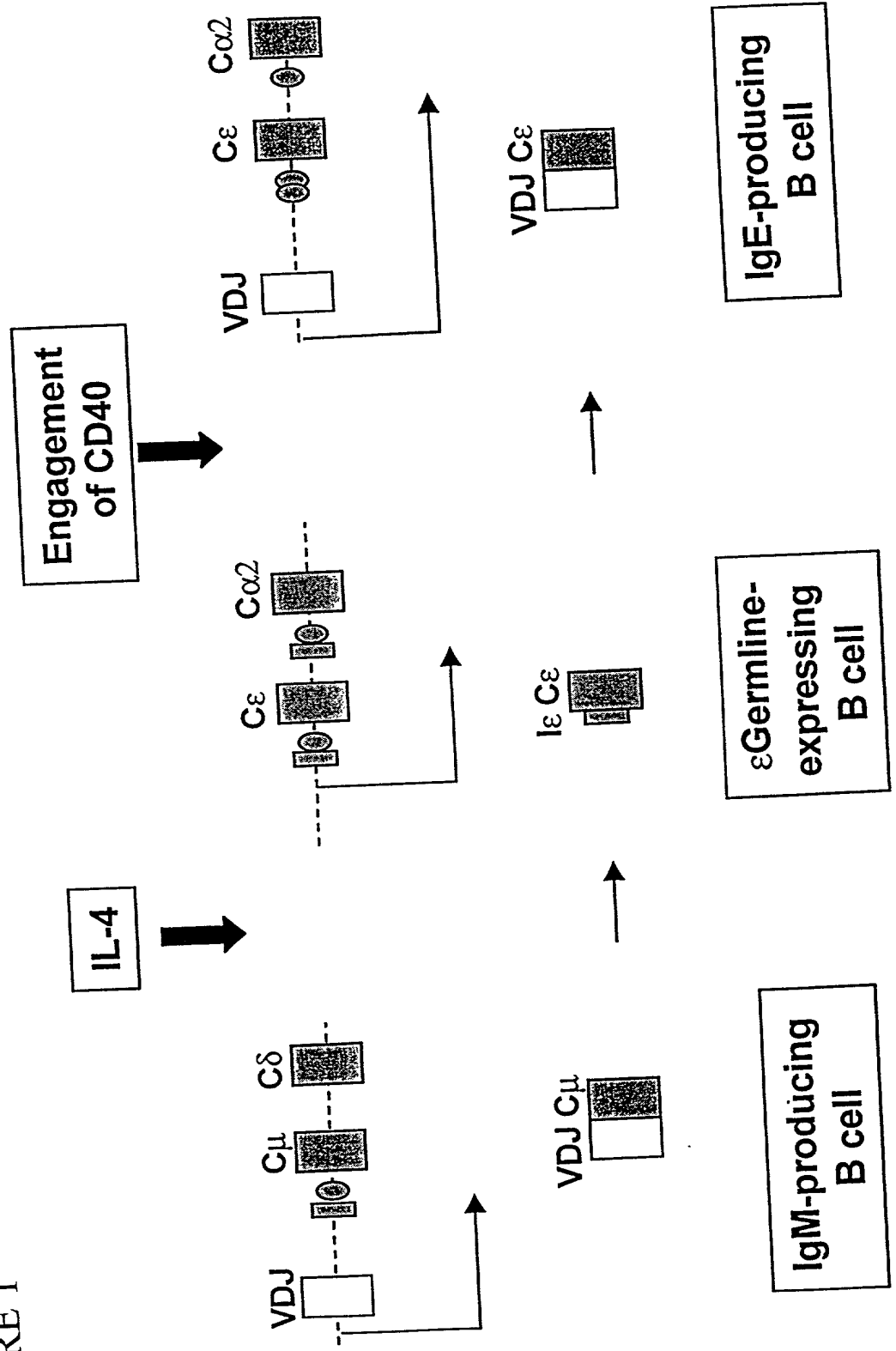


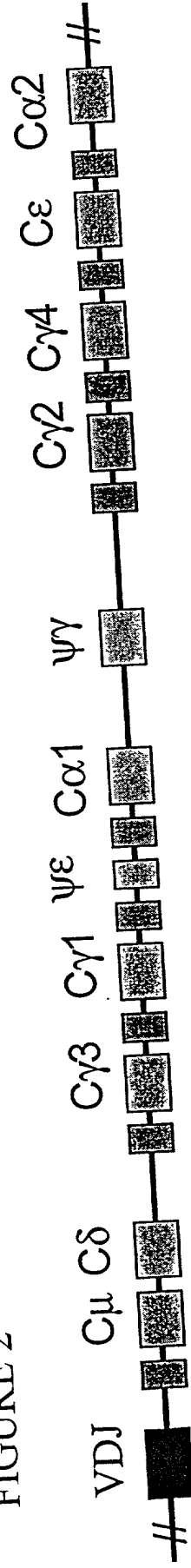
ϵ Germline Transcription and IgE Switching

FIGURE 1

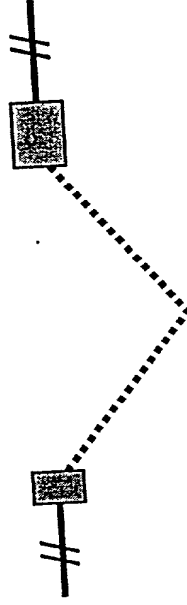


Chromosome 14 Human Heavy Chain Gene Map

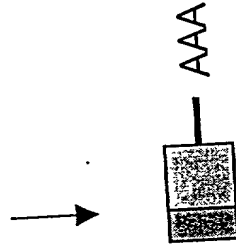
FIGURE 2



Primary Germline Transcript



RNA Processing



Spliced Germline Transcript

■ = | exon

■ = Constant exons

Sequences of RPA Probes for Human Immunoglobulin
Germline Transcripts

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGGGCCGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCCGTGAGGGTGGACCTGCCATGAGGGCCTGCACGCCGGAG
GCCGCCACTCAGCACTGCGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCACGTGAAGCACTACACGAATCCCA
GCCAGGATGTGACTGTGCCCTGCCCAGT
TCCCCACCTCCCCCATGCTGCCACCCCCGACTGTCGCTGCACCGACCGGCC

Germline Ig Epsilon Probe

GGCTCCACTGCCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCATCCACAGGCACCAATGGACGACCCGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCTTG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCGTG

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGCCCCAGGTG
AACGGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTCCACCAAGGGCCCATCGGTCTTCCCC
TGGCACCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACCTCAGGCGCCCTGA
CCAGCGCGTGACACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAAAGTTGAGCC
CAAATCTTGTGACAAAACCTCACACATGCCACCG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACACCAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGGCCCCAGGTGAACGGAGGGGCTTGTCCAGGCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGGTGACGGTGTCGTG
GAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCG
TGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGT
AGATCACAAGCCCAGCAACACCAAGGTG
GACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCACCGTGCCAGCAC
CACCTGTGGCAGGACCGTCA

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGCCCATCGGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACCTCAGGCGCCCTGA
CCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACACCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAGAGTTGAGCT
CAAAACCCCCTTGGTGACACAACCTCACACATGCCCACGGTGCCAGAGCCC
AAATCTTGTGACACACCTCCCCCGTGCC
CACGGTGCCC

FIGURE 3

3 OF 3

Germline Ig Gamma 4 Probe

GGCCAGCACCATGGAAGCCCAAGCGGAGCCAGCACGGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCCACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGCTTCCAAGCCAACAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGGCAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGAGGCCCCAGGTGAACG
GAGGGGCTTGTCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGGGCCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCCTGGTCAAGGA
CTACTTCCCCGAACCGGTGACGGTGTTCGTGGAACCTCAGGCGCCCTGACCAGC
GGCGTGACACCTTCCCGGCTGTCCTAC
AGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG
CTTGGGCACGAAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAA
TATGGTCCCCCGTC

102050-0964860

Sequences of RPA Probes for Human Immunoglobulin Germline Transcripts

Germline Ig Alpha-1 Probe

GGCCTGGGCCGGGGCCAGGGCTCCCTCCCCACAGCAGGCTCTTTGGCAGGCAG
CCAGACGCCCCGTGAGGGTGGACCTGCCA
TGAGGGCCTGCACGCCGAGGCCGCCCACTCAGCACTTCGGGGCCCTCCAGCA
GCTGACCAAGCATCCCCGACCAGCCCCA
AGGTCTTCCCGCTGAGCCTCTGCAGCACCCAGCCAGATGGGAACGTGGTCAT
CGCCTGCCTGGTCCAGGGCTTCTTCCCC
CAGGAGCCACTCAGTGTGACCTGGAGCGAAAGCGGACAGGGCCTGACCGCC
AGAAACTTCCCACCCAGCCAGGATGCCTC
CGGGGACCTGTACACCACGAGCAGCCAGCTGACCTGTTCGGCCACACAGTGC
CTAGCCGGCAAGTCCGTGACATGCCAC

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGTTCGGGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCCGTGAGGGTGGACCTGCCATGAGGGCTTGACGCCCGGAG
GCCGCCCACTCAGCACTGCGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCTCTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCTGCCGGCCACACAG
TGCCACAGACGGCAAGTCCGTGACATGCCAC

Germline Ig Epsilon Probe

GGCTCCACTGCCCGGCACAGAAATAACAACCACGGTTCTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCATCCACAGGCACAAATGGACGACCTGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCCTTG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCTGTG

002050-09624860

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGCTGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGCCCCAGGTG
AACGGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAATCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTECACCAAGGGCCATCGGTCTTCCCC
TGGCACCCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTCCCCGAACCGG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACACACAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGCCCCAGGTGAACGGAGGGGCTTGTCCAGTCCAGCAGCATCAC
TGGAGCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTTCTTGGTCAAGGACT
ACTTCCCCGAACCGG

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGCTGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAATCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGCCATCGGTCTTCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTCCCCGAACCGGTG
ACGGTGTCTGTGGAACCTCAG

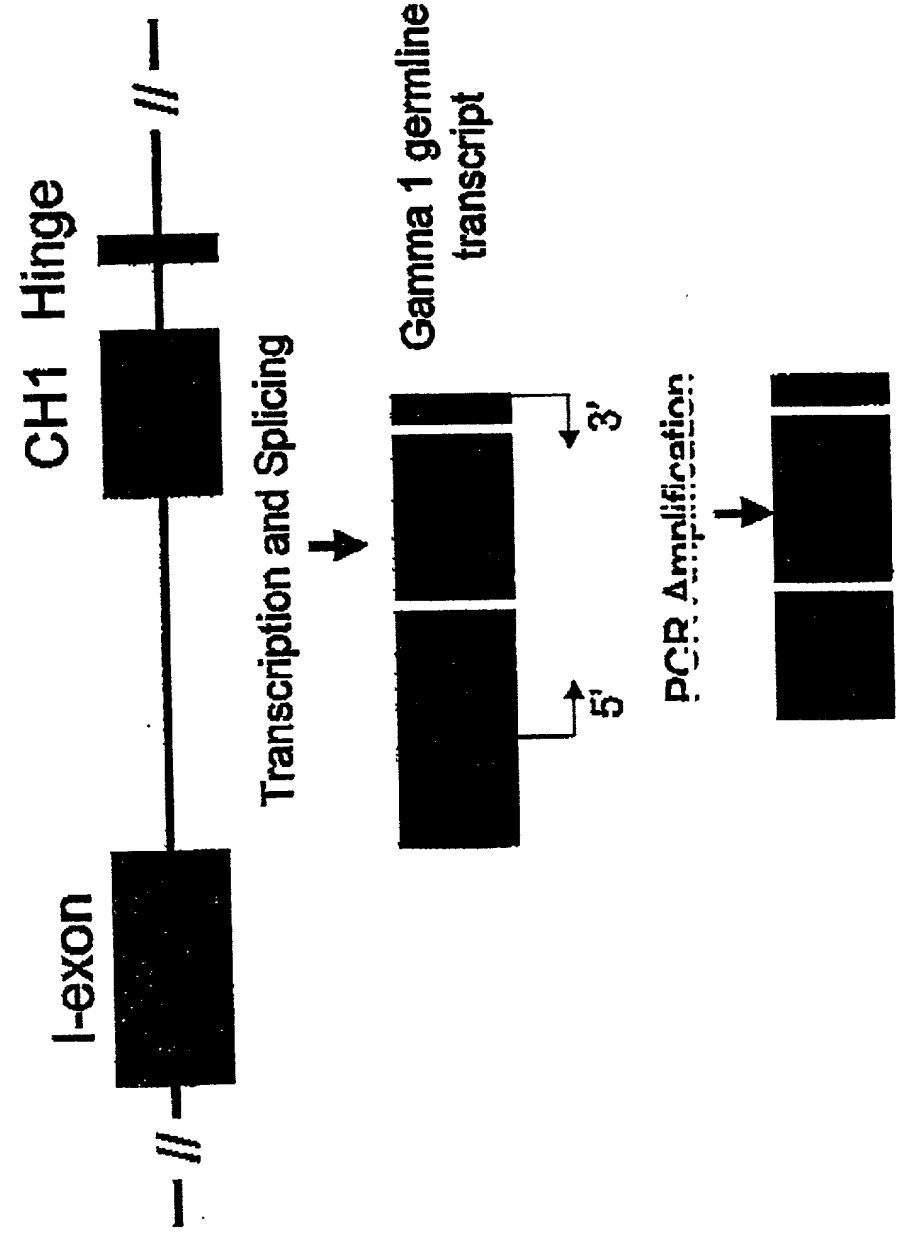
Germline Ig Gamma 4 Probe

GGCCAGCACACATGGAAGCCCAAGCGGAGCCAGCACJGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCCACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGGCTTCCAAGCCAACAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGGACAGAACC GGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGAGACGCCCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGGACCCCTCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCAACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCTGGTCAAGGA
CTACTTCCCCGAACCGG

102050-1964660

FIGURE 6

Gamma 1 Probe



The Gamma 1 5' and 3' Primers amplified a completed probe of 370 BP

RNAse Probe Protection Assay

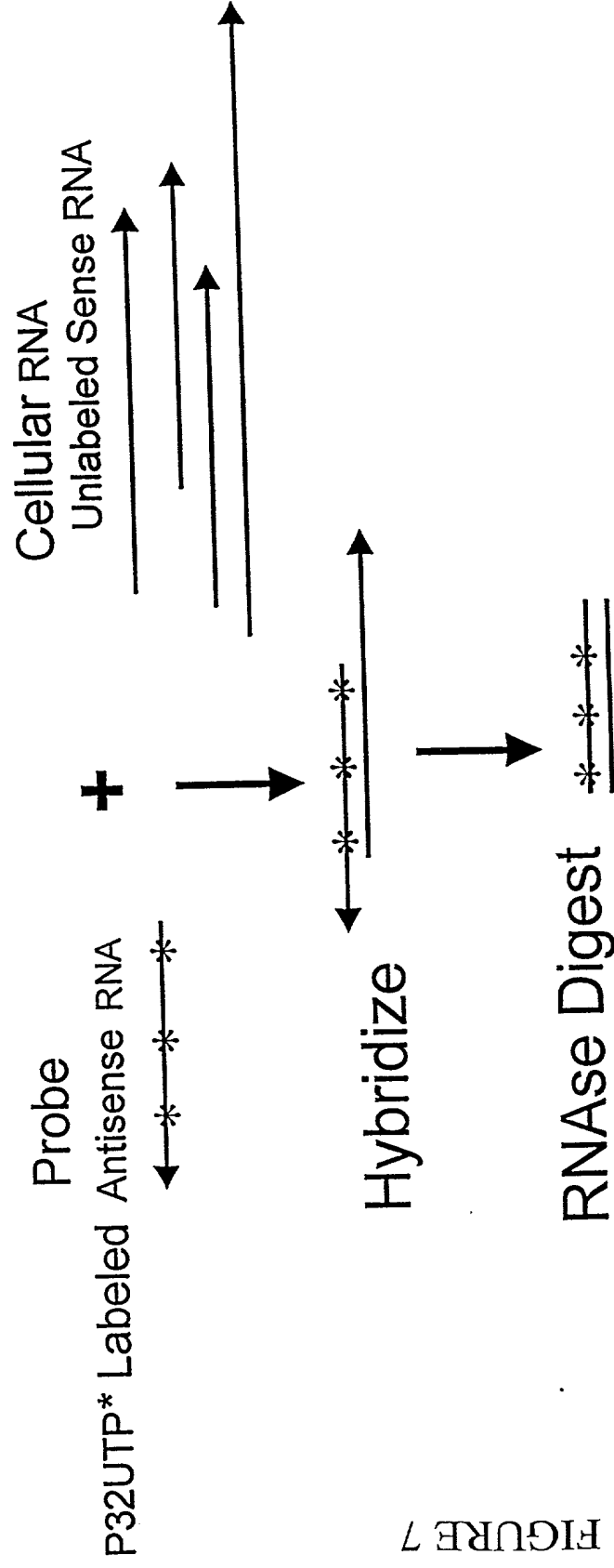
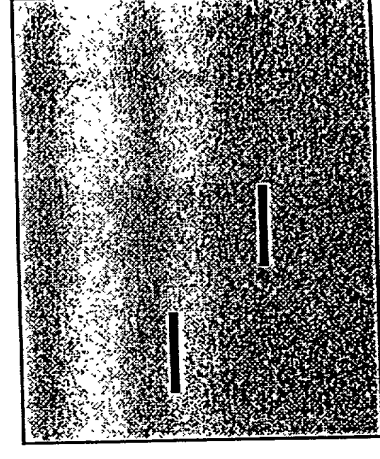


FIGURE 7

Run undigested probe vs digested protected fragment on acrylamide-Urea gel



Undigested Probe

Protected Fragment

Visualize using beta imaging equipment

FIGURE 8

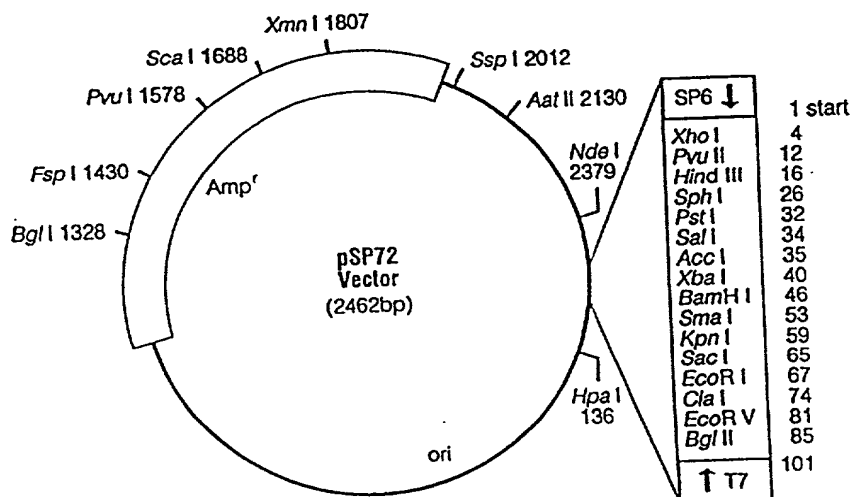


Figure 2. pSP72 Vector circle map and sequence reference points.

1. Sequence reference points:

a. SP6 RNA polymerase transcription initiation site	1
b. T7 RNA polymerase transcription initiation site	101
c. SP6 RNA polymerase promoter	2446-6
d. T7 RNA polymerase promoter	96-118
e. multiple cloning sites	4-90
f. β -lactamase (Amp ^r) coding region	1135-1995
2. Specialized application:
 - a. transcription *in vitro* from dual opposed promoters (For protocol information, please request Promega's Riboprobe® *in vitro* Transcription Systems Technical Manual, #TM016.)
3. The pSP72 and pSP73 Vectors are identical except for the orientation of the multiple cloning region.
4. Blue/white screening for recombinants is not possible with the pSP72 Vector.

Accession Numbers for Germline Transcripts

Alpha - 1

L04541 = I Region Exon
BC005951 = Constant Region Exon

Alpha - 2

L04541 = I Region Exon
AL389978 = Constant Region Exon

Epsilon

X56797 = I Region Exon
J00222 = Constant Region Exon

Gamma - 1

AL122127 = I Region Exon
Z17370 = Constant Region Exon

Gamma - 2

U39934 = I Region Exon
J00230 = Constant Region Exon

Gamma - 3

AL122127 = I Region Exon
X16110 = Constant Region Exon

Gamma - 4

X56796 = I Region Exon
K01316 = Constant Region Exon